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GROWTH PERFORMANCE AND COCCIDIA OCCURRENCE IN PHILIPPINE NATIVE CHICKEN GIVEN DIETS ADDED WITH ORGANIC SELENIUM, PROBIOTICS AND PREBIOTICS

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ABSTRACT

Coccidiosis must be controlled due to its detrimental effect on weight gain and feed conversion of chicken. Routine anticoccidials, however, are unacceptable for organic farming. With the increasing demand for natural and safer food, Philippine native chicken were used to evaluate growth and coccidia incidence when given traditional or alternative anticoccidials. One hundred day-old chicks were brooded for three weeks then ranged until week 12 for the study. Birds were distributed randomly to five treatments (four replicates per treatment, five birds per replicate): Sel: 0.2% organic selenium, Prob: 2% probiotics [*Aspergillus niger, Bacillus subtilis, Enterococcus faecium and Bifidobacterium* spp.], Preb: 0.4% prebiotics [mannan-oligosaccharide], Mad: 0.5% maduramicin (positive control) and Cont: diet without anticoccidia. Feed intake, body weight and average oocysts per gram per bird were recorded between the 0-3, 4-8 and 9-12 weeks. Average daily gain was improved ($p<0.05$) only in Mad between 9-12 weeks. Feed conversion ratio (FCR) was improved ($p<0.01$) by Mad, Sel, Prob and Preb while feed cost per kg were better ($p<0.01$) in Mad, Sel and Preb during the same period compared to Cont. Differences in OPG counts were insignificant but indicate subclinical infection. The study suggests organic selenium- and prebiotic-fed birds can have similar FCR as those given maduramicin at the same feed cost.

Keywords: coccidia, native chicken, oocyst, prebiotic, probiotic

INTRODUCTION

Coccidiosis in chicken is caused by the parasite *Eimeria* spp. The species multiply in the intestinal tract leading to damage and compromising nutrient absorption (McDougald and Fitz-Coy, 2008). Ritzi *et al.* (2014) emphasized that an intact intestinal epithelium prevents entry of potential pathogens resulting to proper nutrient absorption and utilization, thus optimal health and performance of the bird. Coccidiosis is considered to be the most economically important disease in poultry as cost for prophylaxis and treatment reach up to two billion Euros (USD 2.4 billion) at the global level (Dalloul and Lillehoj, 2006). The organism *Eimeria* has developed resistance to drugs not only because of continued use but also due to the parasite's ubiquitous existence in poultry facilities. Oocysts sporulate readily in poultry house litter (Allen and Fetterer, 2002). McDougald and Fitz-Coy (2008) suggested decreasing use of intensive shuttle and rotation programs while combining other control methods to minimize drug resistance.

This study tested organic selenium, prebiotics and probiotics on Philippine native chicken against natural infection of coccidia. Maduramicin was used as positive control. Maduramicin, a polyether ionophore, is highly effective against mixed infections of *Eimeria* spp. (Conway and McKenzie, 2007). Selenium (together with vitamin E) has been shown to improve phagocytic activity in several species. It was suggested that Se protects the

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leucocytes from self-destruction during the phagocytic activity, retaining them at the site of infection, and protecting the host against the pathogenic effects of the parasite (Colnago *et al.,* 1984). Found to be possible antibiotic alternatives, probiotics (live microorganisms) and prebiotics (non-digestible food ingredients selective for good microbes) have also been tested against important pathogens affecting poultry. The proposed modes of action of probiotics and prebiotics are lowering gut pH through lactic acid production, competition for substrates, production of toxic compounds which can inhibit pathogens, competition for attachment sites and stimulation of the immune system (Patterson and Burkholder, 2003; Ferket, 2004; Hajati and Rezaei, 2010).

Range chicken does not seem to be protected from coccidiosis although they are raised in a larger space. In the study of Gari *et al.* in 2008, indigenous chicken showed high prevalence of coccidiosis where sub-clinical coccidiosis was significantly higher than clinical coccidiosis. The prevalence of coccidiosis in Philippine native chicken is unknown. It is believed that although native breeds are more resistant to the disease, it may still affect the chicken's growth performance, thus control measures should be investigated to prevent this. The effect of the addition of organic selenium, probiotics or prebiotics on growth performance of Paraoakan chicken has not been established. Furthermore, the activity of these additives against coccidia in range chicken is still to be proven.

MATERIALS AND METHODS

One hundred Paraoakan day-old chicks were acquired from the National Swine and Poultry Research and Development Center (NSPRDC) - Bureau of Animal Industry (BAI) in Tiaong, Quezon for the study. Birds were distributed randomly to five treatments during the brooding period (day 1-21). Brooder boxes (1.5 sq. m. per 10 chicks) were covered with used newspapers and covered with autoclaved rice hull. Litter was changed as needed and before sample collection for oocyst evaluation. Heater lamps (25 watts/ 10 chicks) were provided as brooders. Temperature was maintained between 32-33°C and reduced by 3°C weekly as recommended (PCARRD, 2006). On Day 21, the birds were divided into four replicates per treatment (five birds per replicate) and were transferred to a ranging area (10 sq. m. per bird). A shed-type housing (0.7 m x 1.7 m) made of local materials was provided per range. Each house has a perch (0.5 m in length) on one side. Catch boards were placed under the perch for fecal collection. Assignment of ranging area followed a completely randomized design (CRD). The birds were gradually introduced to the soil so they were confined inside the house for the first seven days. For the rest of the experimental period, birds were allowed to range during day time while protected inside the house at night.

From Day 1 to Week 6, starter diet was fed to the birds followed by grower/finisher diet from weeks 6 to 12. Diets were based on PCARRD (2006) formulation for broilers. Feed additives were included in the basal diet from day 1 to week 12 as follows: Cont: negative control (basal diet only); Mad: positive control (diet with 0.5% maduramicin, a traditional anticoccidial); Sel: diet with 0.2% organic selenium; Prob: diet with 2% probiotics (combination of *Aspergillus niger, Bacillus subtilis, Enterococcus faecium* and *Bifidobacterium* spp) and Preb: diet with 0.4% prebiotics (mannan-oligosaccharide, MOS) (Table 1). Organic selenium, probiotics and prebiotics are considered as alternative anticoccidials (Elmulsarf and Beynen, 2007). Feeds were given *ad libitum*. Water was made available at all times. Feeders and waterers were placed inside the brooder boxes during the brooding phase and inside the shed-type housing during the ranging period. Grasses and weeds present in the range area were identified. None was found to be detrimental to the birds.

Feed consumption and body weights were obtained and presented by stage, *i.e.* brooding period, 0-3 weeks; then during the ranging period, 4-8 weeks and 9-12 weeks. Coccidia oocyst in terms of oocysts per gram (OPG) evaluation was done from weeks 1 through 12. During the brooding stage (weeks 1-3), the method of Nematollahi *et al.* (2009) was employed while in the ranging period (weeks 4-12), modified McMaster method for OPG evaluation was used (Soulsby, 1982).

Average daily gain (ADG), feed conversion ratio (FCR), feed cost to produce one kg BW and OPG per bird were subjected to analysis of variance (ANOVA) then Scheffe's post hoc (SPSS ver.13) to identify which among treatments had significant differences.

Table 1. Feed composition and nutrient content of basal diet.

Ingredients	Starter (%)	Grower (%)
Copra	0.00	2.03
Corn	54.60	55.82
Coco Oil	1.49	1.01
RBD1	4.96	5.07
Soybean	34.75	31.46
Limestone	1.39	1.22
Monocalcium Phosphorus	1.59	1.93
Vitamin Conc	0.02	0.03
Mineral	0.10	0.10
Salt	0.40	0.41
DL-Methionine	0.10	0.10
Toxin Binder	0.10	0.30
Calculated analysis		
Metabolizable Energy (Kcal/kg)	2903.00	2806.40
Dry Matter	88.78	88.85
Crude Protein, %	20.08	19.01
Crude Fat, %	4.41	4.32
Crude Fiber, %	3.56	3.76
Ash, %	3.65	3.65
Calcium, %	1.05	1.02
Available Phosphorus, %	0.45	0.51
Total Lysine, %	0.20	0.20
Total Methionine, %	0.40	0.40
Total Methionylcystein, %	0.26	0.26
Arginine, %	0.37	0.37
Threonine, %	0.22	0.22
Tryptophan, %	0.05	0.05
Price/ kg (Php)	20.58	21.15

RESULTS AND DISCUSSION

Production performance

Initial body weights of birds were apparently the same for all treatments. Similarly, differences in cumulative and final body weights and feed consumption (g/b/d) were insignificant. The addition of maduramicin, however, improved *(p<0.05)* ADG compared to other treatments between the 9th-12th weeks (Table 2). The results are in contrast to the findings of Awad *et al.* (2009), Kabir *et al.* (2004) and Colnago *et al.* (1984), where final body weights were higher in birds given probiotics, prebiotics or organic selenium. On the other hand, O' Dea *et al.* (2006), gave probiotics via spray, water and in feed and found no differences in body weight, feed conversion nor mortality in birds studied. Similarly, Pelicia *et al.* (2004) used prebiotics and probiotics of bacterial and yeast origin in free range chickens and found no difference in weight gain except in those given in the last stages of study.

In the study of Mountzouris *et al*. (2010), birds given probiotics did not have better weight gains compared the control. The explanation may be "due to the immune stimulatory effects of probiotics—whereby the intake of live microorganisms is aimed to

Table 2. Average daily gain, ADG (g), feed conversion ratio, FCR, feed cost (Php) for 1 kg body weight (BW) and oocysts per gram per bird (OPG) of Philippine native chicken raised in a semi-intensive system.

Treatment*	Control	Maduramicin, 0.5%	Selenium, 0.2%	Probiotics, 2%	Prebiotics, 0.4%
0-3 weeks:					
Ave. daily gain (g)	5.2	5.0	5.1	5.3	5.5
Feed conversion rate	2.2	2.7	2.7	2.5	2.5
Feed cost for 1 kg BW	45.3	55.9	56.1	54.0	52.4
Oocysts per gram/ bird	0.0	0.0	0.0	0.0	0.0
4-8 weeks:					
Ave. daily gain (g)	10.8	12.1	11.9	12.0	11.7
Feed conversion rate	4.5	3.5	3.5	4.1	3.7
Feed cost for 1 kg BW	93.9	73.5	73.7	89.7	78.5
Oocysts per gram/ bird	3024.0	1464.0	1437.0	2133.0	2313.0
9-12 weeks:					
Ave. daily gain (g)**	11.5^{a}	13.8^{b}	13.3^{ab}	13.5^{ab}	13.3^{ab}
Feed conversion rate***	8.9^{b}	6.6^{a}	6.6^{a}	7.0^{a}	6.5^{a}
Feed cost for 1 kg BW***	187.6^{b}	140.9^{a}	140.9^{a}	154.8^{ab}	140.3^{a}
Oocysts per gram/ bird	1421.3	153.0	210.0	675.0	228.8

*Control-basal diet only; Maduramicin-diet with 0.5% maduramicin; Selenium-diet with 0.2% organic selenium; Probiotics-diet with 2% probiotics (combination of *Aspergillus niger, Bacillus subtilis, Enterococcus faecium* and *Bifidobacterium spp*) and Prebiotics-diet with 0.4% prebiotics (mannan-oligosaccharide, MOS).
***Means within the same row with different superscript are different at P< 0.05.*
****Means within the same row with different superscript are different at P< 0.01.*

modulate the gut environment and enhance the gut barrier function via the fortification of the beneficial members of the intestinal microflora, the competitive exclusion of pathogens may have a nutrient and energy cost for the host because live microbes have nutrient requirements for their growth and proliferation." This may also explain why birds given selenium or prebiotics had the same ADG as the control—because energy is required for proper immune function. Patterson and Burkholder (2003) identified many factors contributing to the differences in results with probiotic use (some may also be applicable for prebiotic studies). These are factors which can alter the efficacy of probiotics such as strain(s) of bacteria used, composition and viability and preparation methods. Aside from these, dosage, method or frequency of application, diet, age and condition of birds, housing conditions such as temperature and stocking density and stress experienced by birds, may also contribute to the variation of results.

In terms of FCR though, birds given anticoccidials were better compared to the unmedicated group, Cont ($p<0.01$) only between the 9th -12th weeks. The findings are similar to the study conducted by Ritzi *et al.* (2014), where birds given probiotics in water or in feeds, had the same FCR as the positive control (only, salinomycin was used in their study instead of maduramicin). Like FCR, a highly significant difference ($p<0.01$) in feed cost to produce one kg of body weight (BW) was noted between the 9th and 12th weeks. Feed cost is lower when birds are given additional Mad, Sel or Preb compared to the Cont group. The results suggest that the addition of anticoccidials, traditional or alternative, improves FCR. Furthermore, the use of prebiotics (mannan-oligosaccharide) and selenium can substitute for maduramicin without affecting feed cost.

Coccidia occurrence

Oocysts per gram per bird did not vary among treatments across the examination periods (Table 2). There were no observed oocysts between weeks 0 – 3 in all treatment groups. Numbers peaked between weeks 4 – 8 with an average OPG per bird of 1,437 – 3,024. Birds given selenium had the lowest OPG while Cont. had the highest. Between weeks 9 – 12, average OPG per bird were reduced to low counts from 153 (Mad) – 1,421 (Cont). There are many factors which can influence oocyst shedding and the time when such are released in the fecal matter, thus resulting to the variation in the OPG. According to Bumstead *et al.* (1991), the amount of oocysts discharged from infected chicken depends on the dose of oocysts ingested and the immunological status acquired from pre-exposure. This means that greater OPG discharged in the feces suggests that greater number of oocysts was ingested. Although this may sound harmful, initial exposure to the parasite is important for priming the immune system against coccidia. Chapman *et al.* (2010) stressed that different anti-coccidials have varying effects on oocyst shedding and cycling, which is essential for adequate acquired coccidial immunity. Those that limit shedding of the parasite may not elicit appropriate immune response. Clearly, such factors contributed to the variation in OPG counts among treatments in different stages.

Studies which used selenium, probiotics and prebiotics have varying results on oocyst shedding. Ritzi *et al.* (2014) found out that birds intermittently given high dose of probiotics in feed and in water shed fewer oocysts than the chicken in the positive control group, while in the findings of Dalloul *et al.* (2003), weight gain and oocysts shedding were not improved by a *Lactobacillus*-based probiotic in the face of *Eimeria tenella* infection, but antibody titers against the parasite were higher in birds given probiotics. Similarly, although there were no decreased cecal lesions and improved growth performance in birds challenged with *Eimeria*, the use of MOS was found to suppress coccidiosis (Elmusharaf *et al.,* 2006). While it is a fact that coccidiosis is hard to control because of the intensive rearing conditions, raising birds in semi-intensive system also has disadvantages. As observed in this study, the wider space where birds are allowed to range also allows greater area for oocyst dispersal. Birds also have a greater chance of ingesting oocyts as

they are constantly in direct contact with the soil.

The average OPG counts (1,300 – 2,300) noted by Gari *et al.* (2008) in indigenous chicken in Ethiopia, is similar to this study. However, the clinical signs of coccidiosis such as depression, ruffled feathers, diarrhea, and/ or blood-mixed droppings were not noted in this study. Although management difference may account for the susceptibility of Ethiopian strains, the addition of selenium, probiotics or prebiotics probably alleviated the effects of infection. In the study of North and Bell (1990), the presence of at least, 10,000 specific *Eimeria* species is required to show the signs and symptoms of coccidiosis. Since all the OPG counts recorded were below 10,000, this may be quantified as a subclinical coccidial infection.

OPG counts between 9th and 12th weeks were not different among treatments but FCR was. Those given anticoccidials had better FCR. The highly significant difference in FCR suggests that the addition of anticoccidials, may it be conventional or alternative, improves FCR in the presence of subclinical coccidial infection. As Williams (1999) said, subclinical coccidiosis may reduce weight gain and feed efficiency.

CONCLUSION

Compared to Cont, only maduramicin improved ADG between the 9-12 weeks ($p<0.05$). In the same observation period, FCR was improved by the addition of maduramicin, organic selenium, probiotics or prebiotics ($p<0.01$). Feed cost to produce a kg of BW was cheaper in Mad, Sel and Preb ($p<0.01$) compared to Cont and Prob. Differences in OPG counts per bird were insignificant but suggest subclinical infection. Because subclinical coccidiosis affect weight gain and feed efficiency, the addition of anticoccidials (maduramicin, organic selenium, probiotics or prebiotics) possibly reduced the negative effects of infection.

REFERENCES

Allen PC and Fetterer RH. 2002. Recent advances in biology and immunobiology of *Eimeria species* and in diagnosis and control of infection with these coccidian parasites of poultry. *Clin Microbiol Rev* 15 (1): 58–65.

Awad WA, Ghareeb K, Abdel-Raheem S and Böhm J. 2009. Effects of dietary inclusion of probiotic and synbiotic on growth performance,organ weights, and intestinal histomorphology of broiler chickens. *Poult Sci* 88: 49–55.

Bumstead N, Millard BM, Barrow P and Cook JKA. 1991. Genetic Basis of Disease Resistance in Chickens. In: Owen, F.B. and Ax Ford RFE. (eds.): *Breeding for Disease Resistance in Farm Animals*. Oxfordshire, UK: CAB International, pp. 10-21.

Chapman HD, Jeffers TK and Williams RB. 2010. Forty years of monensin for the control of coccidiosis in poultry. *Poult Sci* 89: 1788.

Colnago GL, Jensen LS and Long PL. 1984. Effect of selenium and vitamin E on the development of immunity to coccidiosis in chickens. *Poult Sci* 63 (6): 1136-43.

Conway DP and McKenzie ME. 2007. *Poultry coccidiosis.* Diagnostic and testing procedures. 3rd ed. Ames, IA, USA: Blackwell Publishing. p 118.

Dalloul RA and Lillehoj HS. 2006. Poultry coccidiosis: recent advancements in control measures and vaccine development. *Expert Rev Vaccines* 5 (1): 143-63.

Dalloul RA, Lillehoj HS, Shellem TA and Doerr JA. 2003. Enhanced mucosal immunity against *Eimeria acervulina* in broilers fed a *Lactobacillus*-based probiotic. *Poult Sci* 82: 62–66.

Elmusharaf MA and Beynen AC. 2007. *Coccidiosis with emphasis on alternative anticoccidial treatments*. Retrieved on 26 July 2011 from http://www.researchgate.net/publication/267683539_Coccidiosis_in_Poultry_with_Emphasis_on_Alternative_Anticoccidial_Treatments.

Elmusharaf MA, Bautista V, Nollet L and Beynen AC. 2006. Effect of a mannanoligosaccharide preparation on *Eimeria tenella* infection in broiler chickens. *Int J Poult Sci* 5 (6): 583-588.

Ferket PR. 2004. Alternatives to antibiotics in poultry production: Responses, practical experience and recommendations. Nutritional biotechnology in the feed and food industries: *Proceedings of Alltech's 20th Annual Symposium*, Kentucky, USA, pp 56-57.

Gari G, Tilahun G and Dorchies Ph. 2008. Study on Poultry Coccidiosis in Tiyo District, Arsi Zone, Ethiopia. *Int J Poult Sci* 7 (3): 251-56.

Hajati H and Rezaei M. 2010. The application of prebiotics in poultry production. *Int J Poult Sci* 9 (3): 298-304.

Kabir SML, Rahman MM, Rahman MB, Rahman MM and Ahmed SU. 2004. The dynamics of probiotics on growth performance and immune response in broilers. *Int J Poult Sci* 3: 361–364.

McDougald LR, and Fitz-Coy SH. 2008. Protozoal infections. In: *Diseases of Poultry*. Y. M. Saif, ed. Ames, IA, USA: Blackwell Publishing. Pp. 1067–1117.

Mountzouris KC, Tsitrsikos P, Palamidi I, Arvaniti A, Mohnl M, Schatzmayr G, Fegeros K. 2010. Effects of probiotic inclusion levels in broiler nutrition on growth performance, nutrient digestibility, plasma immunoglobulins, and cecalmicroflora composition. *Poult Sci* 8: 58–67.

Nematollahi A, Moghaddam G and Pourabad RF. 2009. Prevalence of Eimeria species among broiler chicks in Tabriz (Northwest of Iran). *Mun.Ent. Zool*. 4: 53-58.

North OM and Bell DD. 1990. *Commercial chicken production*. 4th ed. New York, USA: Chapman and Hall Publishers. Pp. 826-828.

O' Dea EE, Fasenko GM, Allison GE, Korver DR, Tannock GW and Guan LL. 2006. Investigating the Effects of Commercial Probiotics on Broiler Chick Quality and Production Efficiency. *Poult Sci* 85:1855–1863.

Patterson JA and Burkholder KM. 2003. Application of prebiotics and probiotics in poultry production. *Poult Sci* 82:627–631.

PCARRD. 2006. The Philippines Recommends for broiler production. Series No. 10-C. Revised. Los Baños, Laguna.

Pelicia K, Mendes AA, Saldanha ESPB, Pizzolante CC, Takahashi SE, Moreira J, Garcia RG, Quintero RR, Paz ICLA and Komiyama CM. 2004. Use of prebiotics and probiotics of bacterial and yeast origin for free-range broiler chickens. *Brazilian J Poult Sci* 6 (3): 163 – 169.

Ritzi M, Abdelrahman W, Mohnl M and Dalloul RA. 2014. Effects of probiotics and application methods on performance and response of broiler chickens to an Eimeria challenge. *Poult Sci* 93: 2772–2778.

Soulsby EJL. 1982. *Helminths, arthropods and protozoan's of domesticated animals*, 7th ed. London, UK: Bailliere Tindall. p. 766.

Williams RB. 1999. A compartmentalised model for the estimation of the cost of coccidiosis to the world's chicken production industry. *Int J Parasitol* 29: 1209-1229.